

Based on this finding the invention provides a method for the specific identification of differentially expressed DNA-sequences comprising the use of Differential Display Reverse Transcription PCR, in which one set of partially or completely degenerated primers specific for the target gene is used. One major limitation of the conventional RNA display strategy is the lack of specificity of the method. In the aim to increase this specificity, the inventors in their search for other adhesion molecules used degenerated primers targeting the sequence encoding molecules with C₂ domains. This was achieved by the alignment of C₂ domains of several Ig Sf adhesion molecules, and the identification of a linear amino-acid consensus, surrounding the cysteine residue participating to the C₂ domain structure: Y-(RQYS)-C-x-A-S-N-x₂-G (SEQ ID NO.: 22). In a more general sense, this approach can also be used in the search for other sequences in which the reverse translation of one or more of the most frequent consensus sequences is used to design the degenerated primers used for differential display.

At page 5, kindly replace the paragraph beginning at line 21 with the following paragraph.

In addition to using the sequence information of the two CRAM proteins disclosed herein for identifying other members of the family in other species, like humans, the two proteins and their corresponding family members can also be used for the preparation of derived molecules, such as antibodies directed against the (poly)peptides of the invention, or recombinant equivalents of the proteins, optionally in soluble form, or peptides comprising at least part of the amino acid sequence of the polypeptides. Suitable parts of the amino acid sequence are especially the extracellular domains: VC₂, and the membrane proximal cytoplasmic sequence: A-[Y,Q]-[R,S]-[R,K]-G-[C,Y]-F (amino acids 266-272 of SEQ ID NO.:13 or amino acids 261-267 of SEQ ID NO.: 14).

At page 9, kindly replace the paragraph beginning at line 5 with the following paragraph.

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Fig 8: (A) nucleotide and deduced amino acid sequence of the Confluence Regulated Adhesion Molecule 1 (CRAM-1) cDNA. The putative hydrophobic signal peptide (first) and transmembrane region (second) are underlined. Predicted N-glycosylation sites (strikeout), cysteines likely to form disulfide bonds (brackets) and Ser/Thr/Tyr residues of possible phosphorylation sites (bold) are indicated. (B) Structural model for murine CRAM-1 protein. Extracellular part showing a VH and a C2 like Ig domain with two putative N-linked glycosylation sites. The arrow points to the region targeted by the partially degenerated primers (YYCXAS1) (SEQ ID NO.:20) used in the Targeted Differential Display.

At page 22, kindly replace the paragraph beginning at line 2 with the following paragraph.

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The regulation of genes in endothelial cells depends on their environment. The present invention was directed to the identification of genes that undergo regulation upon the contact of endothelium with tumor cells. For this purpose, an in vitro assay was developed using the co-culture of melanoma tumor cells (B16) with an endothelioma cell line (t-end). Total RNA extracted from the mix was used as a template to prepare cDNA submitted to a differential PCR screen. The cDNA obtained from the endothelial or melanoma cells cultured on their own were used as controls. The three different patterns were compared to identify the transcripts regulated by the co-culture condition. To limit the analysis to the sequences encoding for cell surface molecules of Ig superfamily, partially degenerated primers were used that target the sequence surrounding the C-terminal cysteine of C2 domains in Ig superfamily molecules. The most reproducible pattern of PCR products was

obtained using primers that encode the sequence YYCxAS1 (Fig 7A, SEQ ID NO.: 20). This improved method of RNA display technique was named TDD for "Targeted Differential Display".

At page 33, kindly replace the paragraph beginning at line 11 with the following paragraph.

The new screening strategy, named Targeted Differential Display (TDD), has proved to be an efficient technique in selectively amplifying cDNA of interest. TDD successfully exploited the use of partially degenerated primers to confer selective targeting to the conserved region, Y(Y/Q/R)CXAS (SEQ ID NOS.: 18-20), of C2 like Ig domains. Repeated experiments lead to reproducible display patterns. Out of 16 differentially expressed transcripts, three correspond to genes with significant homology to conserved Ig sequences. This increase in specificity manages to overcome the major difficulties in the already known techniques of classical RNA fingerprinting and differential display. RNA fingerprinting has long been used for the identification of differentially expressed genes. However, due to the sequence specific primers employed, this method detects only the transcripts of selected and already known proteins. On the other hand, RNA display employs random primers and involves the non-specific amplification of transcripts. The aim in this case is to pinpoint any differences in mRNA levels between two biological systems, which are submitted to comparison. TDD is an advanced screening method that combines the specificity of RNA fingerprinting with the degeneracy of Differential RNA Display resulting in selectivity. Due to the targeting of related transcripts, this technique significantly reduced the time needed for screening. The identification of new members of specific protein families, therefore, becomes possible. This is a